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14. ABSTRACT

Malaria has proven refractory to conventional immunization approaches. We are exploring a novel route to induction of anti-malaria immunity: adeno associated virus (AAV) vectored transfer of genes encoding known protective monoclonal antibodies (MAbs) to whole animals. Using a specific technology originally applied to expression of HIV antibodies, we demonstrated that mice can be protected from *Plasmodium falciparum* infection by antibodies against circumsporozoite protein, an antigen found on the surface of the form of the parasite injected by mosquitoes. This project has two specific aims:
1. Identification of optimal MAbs by construction of additional vectors and assessments of protective efficacy in mice, and 2. Tests of protective efficacy of these MAbs, delivered by AAV vectors, in a non-human primate (*Aotus nancymaae*) model of *P. falciparum* infection. In this period, five MAb vectors have been constructed and fully or partially characterized in mice (See below). Two of these exhibit efficacy comparable to the MAb used in our original studies and are undergoing sequence optimization that is expected to enhance efficacy. NHP trials have been hampered by technical difficulties in reproducing the published challenge protocols (see below), and efforts on that aim are currently directed toward validating the challenge assay.

15. SUBJECT TERMS
Malaria, monoclonal antibody, immunization, vaccine, gene transfer, adeno associated virus, AAV, *Plasmodium falciparum,* sporozoite murine challenge model, non-human primate challenge model, *Aotus*

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1. Introduction. Malaria is caused by parasites of the genus *Plasmodium* and is responsible for about 500,000 deaths per year, mostly in sub-Saharan Africa and mostly induced by infection with *P*. falciparum. In addition to the burden it imposes on residents of endemic areas, malaria poses a significant threat to US service personnel serving in Africa and other malaria-endemic areas. An effective vaccine would be of enormous value in relieving the toll exacted by malaria. However, extensive efforts to develop malaria vaccines using conventional approaches have been largely unsuccessful and no satisfactory malaria vaccine exists. The long-term objective of this project is to assess the promise of a novel immunization technology termed vectored immunoprophylaxis (VIP) in inducing protective immunity to malaria. VIP employs adeno associated virus (AAV) vectors to deliver genes encoding monoclonal antibodies (MAbs) to animals. Mice transduced by VIP vectors that encode monoclonal antibodies directed against the *P. falciparum* circumsporozoite protein (CSP) rapidly develop high serum levels of the MAb and are protected from experimental infection by a transgenic rodent parasite that expresses *P. falciparum* CSP. This project will assess in more depth the potential of VIP technology in malaria immunization. It has two specific aims: 1. to use the murine challenge model to identify additional MAbs with potential in the VIP system and optimize their expression *in vivo*, and 2. to test the most promising MAbs for protective efficacy in a non-human primate model of *P. falciparum* infection that employs *Aotus nancymaae* new-world monkeys.

2. Keywords: Malaria, monoclonal antibody, immunization, vaccine, vectored immunoprophylaxis, gene transfer, virus vector, adeno associated virus, AAV, *Plasmodium falciparum,* sporozoite, murine challenge model, non-human primate challenge model, *Aotus*

3. Accomplishments.

A. Major Goals	**Timeline (months)**	
	Projected	
Completed(%)		
Goal 1: VIP vector development		
1. Prepare, purify and sequence new MAbs	1-12	75
2. Construct first-round vectors	1-18	50
3. Optimize MAb expression in new vectors	3-18	25
Milestone: Selection of candidates for mouse experiments.	12-18	50
Goal 2: Evaluate candidate vectors in mice		
1. Local IRB/IACUC Approval	Completed	
2. Assess protection by new VIP vectors; IV challenge	6-30	20
3. Assess protection by new VIP vectors; mosquito bite challenge	12-30	30
4. Determine mouse dose-responses; mosquito bite challenge	18-30	0
5. Assess protection by vector pairs; mosquito bite challenge	18-30	50
Milestones: Selection of VIP vectors for *Aotus* studies.	12, 18-24	50
Goal 3: Determine *Aotus* dose response	7-18	
1. Local IRB/IACUC Approval	Completed	
2. Dose response in *Aotus*	Completed	
Goal 4: *Aotus* challenge 1 (mAb 2A10)	13-30	0

Goal 5: *Aotus* challenge 2 (mAbs TBD)	19-36	0
Milestone: Selection of vectors potential clinical trials.	36	0

B. What was accomplished under these goals

Goal 1: VIP vector development*. 1. Prepare, purify and sequence new MAbs.* The amino acid sequence of a single MAb (of seven that we plan to characterize; see below) remains to be sequenced. This information required for vector construction. No difficulties are anticipated in this process.

2. Construct first-round vectors. We will test seven MAbs in mice: four directed against the *P. falciparum* CSP repeat, one for a second conserved region in CSP, and two directed against CelTOS, a protective antigen described by E. Angov of WRAIR. Two additional CSP repeat MAbs were characterized before CDMRP funding began. Of the seven new MAbs, one remains to be sequenced (see above) and vectors have been constructed for 5 more; 4 of those during this funding period. A vector for the final MAb is currently under construction.

3. Optimize MAb expression in new vectors. Mouse tests of three vectors revealed that they direct only modest levels of serum MAb expression, substantially lower than those of previously-characterized MAbs. The amino acid sequence of the framework portions of the MAb variable regions can affect expression levels, and framework sequences from one high-expressing MAb are being incorporated into the vector that will encode the final MAb, currently under construction. If that approach enhances expression, at least two of the previously-constructed vectors will be modified to include the better-expressed framework and tested for level of expression in mice. Importantly, while it is clear from our published data that high expression levels enhance protective efficacy, it remains likely that extravagant levels of expression of a potent MAb will not be needed to confer protection. Therefore, pursuit of enhanced MAb expression is considered an important but not essential element of the project.

Goal 2: Evaluate candidate vectors in mice. *1. Assess protection by new VIP vectors; intravenous (IV) challenge.* Three MAbs that have been assessed for protective efficacy by both IV injection of sporozoites and exposure to infected mosquito bites. One is protective in both assays and one is protective in neither. The remaining MAb protects in mosquito bite challenge, but not IV challenge. Because mosquito bites represent the route of natural infection and seem in addition to provide a more sensitive indication of protection, use of IV challenge as a measure of efficacy for new vectors has been suspended. However, mosquito bite challenges may not permit unambiguous discrimination of efficacy among candidate vectors for the NHP studies. In that case, IV challenges will be undertaken to provide additional data for use in vector selection for NHP challenge.

3. Assess protection by new VIP vectors; mosquito bite challenge. Mosquito bite challenge experiments have been completed or done once (of two planned independent repetitions) for two new MAbs. Both of these, which are expressed at modest levels from current vectors (see above), provided protection in a proportion of immunized mice (50-70%) comparable to the highly-expressed 2A10 MAb whose characterization was completed prior to this award. Both of these MAbs therefore remain candidates for use

in NHP studies. Because protection by these MAbs was observed at levels of expression somewhat lower than that of 2A10, we expect that if framework optimization (see above) is successful, modified versions of these MAbs will prove leading candidates for NHP studies.

4. Determine mouse dose-responses; mosquito bite challenge. These experiments will be done when vectors of higher potency than the existing 2A10 vectors are identified, as the dose range of these studies are currently limited by the volumes of virus preparations that can be used in mice.

5. Assess protection by vector pairs; mosquito bite challenge. These studies are based on the hypothesis that MAbs targeting distinct antigenic epitopes (on the same of different proteins) may act synergistically to confer better protection than either MAb alone. Studies of one pair of MAbs have been completed. This study included the 2A10 MAb and MAb 5D5**.** This pair was chosen because the two MAbs target distinct epitopes: the CSP central repeat (2A10) and a conserved epitope in CSP that lies near the site of a proteolytic cleavage that is required for cell invasion by sporozoites. 2A10 is protective in about 70% of animals, while 5D5 is not protective alone. The combination has efficacy indistinguishable from that of 2A10 alone, indicating that in this case, no synergy occurs. A second pair (2A10 and one of the CelTOS MAbs) will be tested when tests of the individual efficacies of the CelTOS MAbs are completed.

Goal 3: Determine *Aotus* dose response. 1. *Local IRB/IACUC and ACURO Approva*l has been obtained.

2. *Dose response in Aotus.* These studies have been completed for MAb 2A10 with two points based on literature values for related vectors. Surprisingly, the lower dose tested (2×10^{12} genome copies [GC] per monkey) proved to yield serum MAb levels equal to that of the higher dose (10^{13} GC/monkey). Thus, the system seems to be saturated with respect to the inoculum of AAV. Since the amount of virus needed to perform the subsequent planned studies in *Aotus* at the lower dose is easily obtained and the low dose is well-tolerated by the monkeys, we won't at this time explore reduced doses. Ultimately, it may be desirable to test lower doses to determine the minimum amounts of vector that produces a protective response in preparation for clinical trials. This, however must await the successful development of the challenge system (see below) and will not be attempted at this time.

Goal 4: *Aotus* challenge 1 (mAb 2A10). Significant difficulties encountered in implementing the challenge in the *Aotus* monkeys have prevented initiation of tests of MAb 2A10. The difficulties and progress in overcoming them are described in detail in Section 5, below.

Goal 5: *Aotus* challenge 2 (mAbs TBD). Not yet underway.

C. Opportunities for training and professional development. Nothing to report

D. How results were disseminated Nothing to report

E. Plans for next reporting period

1. Vectors encoding the remaining MAbs will be completed and tests of efficacy in mice will be performed. The remaining planned multiple-MAbs test will be done. No difficulties are anticipated in these efforts

2. The *Aotus* challenge system will be implemented. Issues with this system are described in Section 5, below.

4. Impact. Nothing to report.

5. Changes/Problems. A protocol for the assessment of pre-erythrocytic vaccine efficacy in *Aotus nancymaae* monkeys have been published. Briefly, splenectomized *Aotus* monkeys are challenged by IV injection of malaria sporozoites, and are then assessed for development of parasitemia. Protection reduces the proportion of the challenged *Aotus* that become parasitemic. We have encountered difficulties in implementing this protocols that have precluded initiation of the next phase of the project, challenges in *Aotus*. We are currently working urgently to address these difficulties.

Reports in the literature indicate that few *P. falciparum* isolates are suitable for sporozoite infection of NHPs. The most efficient of these is a strain called Santa Lucia. St. Lucia is not, however, a commonly used strain and some difficulty was encountered in obtaining it. Eventually, the strain was located at the NIH and an aliquot was provided by Thomas Wellems.

As received, St. Lucia grew in human RBCs in culture and gametocyte production could be induced by methods routinely used in the Johns Hopkins Malaria Research Institute (JHMRI) parasite core. *An stephensi* mosquitoes fed on the gametocyte cultures produced oocysts, but did not produce sporozoites, preventing sporozoite challenge.

It is not uncommon for malaria strains passed in culture to lose infectivity for mosquitoes, but infectious parasites can sometimes be selected from such populations by passage through animals. Therefore, it was decided to attempt to rejuvenate our St Lucia isolate by growth through a complete life cycle in *Aotus* (Figure 1). In this effort, two splenectomized *Aotus* were inoculated with blood-stage parasite cultures produced *in vitro*. Both *Aotus* became parasitemic and both developed gametocytes. *An stephensi* mosquitoes were fed

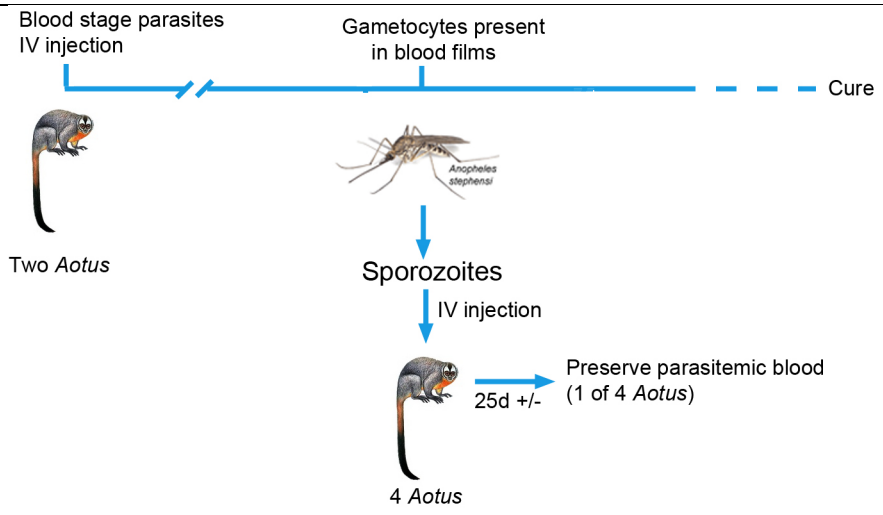

Figure 1. Passage of Saint Lucia *P. falciparum* parasite through *Aotus*.

on both monkeys. These mosquitoes developed oocysts and sporozoites, and multiple sporozoite preparations were made and injected into four naïve splenectomized *Aotus*. One of the four sporozoite-inoculated monkeys developed parasitemia. Blood was drawn from this animal and aliquots were preserved. Those aliquots, when amplified in culture, are infectious for mosquitoes and infected mosquitoes produce sporozoites. Thus, at least sporozoite production was restored by animal passage.

Cultures of the sporozoite-competent St Lucia strains are currently being prepared for mosquito feeding. Sporozoites will be dissected from the mosquitoes, and injected IV, per the published challenge protocol, into five naïve *Aotus* (planned for November 6, 2017). The outcome of that infection will determine whether the challenge of animals transduced with the 2A10 MAb vector can proceed.

6. Products Nothing to report

7. Participants and collaborating Organizations.

Personnel

Gary Ketner Ph.D. No change

Robert J. Adams. DVM. No change

Renuka Elizabeth Joseph, ScM. Left institution for PhD program

Brendan Dolan. Graduated

Gloria Shin, PhD.
Postdoctoral Fellow
12 person-months
Design and conduct of experiments in culture and in mice; analysis of *Aotus* samples for mAb expression, construction of VIP vectors, analysis of challenge results
Funding support: This award

Changes in active other support. Nothing to report

Organizations

PATH/MVI
2201 Westlake Avenue, Suite 200, Seattle, WA 98121
Furnished anti-CSP monoclonal antibody sequences

Walter Reed Army Institute of Research
503 Robert Grant Avenue
Silver Spring, MD 20910-7500
Furnished anti CelTOS monoclonal antibodies on a collaborative basis

Leidos
5202 Presidents Court
Frederick, MD 21703
Furnished 5D5 MAb sequence

8. Special reporting requirements. None

9. Appendices. None